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(54) Title: PEPTIDE ANALOGS AND THEIR USE AS HAPTENS TO ELICIT CATALYTIC ANTIBODIES

(57) Abstract

Haptens capable of eliciting antibodies which can catalyze chemical reactions, comprising a hapten or a hapten and a suitable carrier molecule are disclosed. In particular, spiro[4.4]nonane containing dipeptide analogs, which mimic both a torsionally-distorted peptide ground state and the transition state for peptide bondhydrolysis, are described, along with methods of their synthesis and their coupling with amino acids of the D-configuration are described. Antibodies which are catalytically active for chemical reactions, in particular, the cleavage or formation of a selected peptide bond, and which are elicited by such antigens are disclosed as well as methods for producing the antibodies and methods for catalyzing the cleavage or formation of a petpide bond in a molecule.

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PEPTIDE ANALOGS AND THEIR USE AS HAPTENS TO ELICIT CATALYTIC ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of copending U.S. Application Serial No. 08/134,492, filed October 8, 1993, which in turn is a continuation of U.S. Application Serial No. 07/700,210, filed June 12, 1991 and now abandoned, which is a continuation-in-part of U.S. Application Serial No. 07/190,271, filed May 4, 1988 and now abandoned, the contents of which applications are hereby expressly incorporated by reference into the present disclosure.

GOVERNMENT RIGHTS STATEMENT

This invention was made with government support under GM-39758 awarded by the National Institutes of Health and MCB-8958239 awarded by the National Science Foundation. The government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates generally to antibodies, and antigens, haptens and immunogens capable of eliciting such antibodies, which include a paratope that binds to and thereby stabilizes a transition state in the cleavage or formation of a peptide bond linkage so that the cleavage or formation is catalyzed by the antibodies. Said paratope also binds to and destabilizes the substrate ground state.

BACKGROUND OF THE INVENTION

Several publications are references in this application by Arabic numerals within parentheses. Full citation for these references are found at the end of the specification immediately preceding the claims. The references more fully describe the state-of-the-art to which this invention pertains as well as certain aspects of the invention itself.

There are numerous enzymes which have been identified as capable of catalyzing various chemical

reactions. Similarly, it has been discovered that antibodies can be elicited to catalyze a variety of chemical reactions (U.S. Patent No. 4,888,281 to Schochetman et al., issued December 19, 1989). It is well known that antibodies and enzymes share a fundamental similarity in that both are specialized proteins that bind to other molecules. However, there are important physiological differences between antibodies and enzymes.

Antibodies typically bind to a molecule or antigen so that the antigen is marked as foreign to the organism that produced the antibody. The binding of the antibody to the antigen enables the antigen to be removed from the organism. Enzymes are biological catalysts which bind a molecule in such a way that the activation energy of a reaction involving a molecule or substrate is lowered, thereby increasing the rate of the reaction.

Consistent with this distinction, Linus Pauling hypothesized that antibodies bind molecules in their ground states most strongly while enzymes bind molecules in higher energy states most strongly.

Pauling attempted to explain the mechanism of enzyme catalysis based upon such binding. During the course of the chemical reaction, the reactants undergo one or more transitions through intermediate structures or transition states which are energically less favorable than either the reactant or the product. The hydrolysis reaction of a peptide linkage or an ester bond in an aqueous medium passes through a tetrahedral carbon transition state. In the transition state, a tetrahedral carbon atom is bonded to: a carbon atom of the acid portion of the peptide linkage or ester bond; two oxygen atoms, one corresponding to the carbonyl group and the other corresponding to a hydroxyl ion or water molecule of the medium; and either the oxygen atom of the alcohol portion of an ester or the nitrogen atom of the amine portion of the peptide linkage. The transition state can

be neither isolated nor detected since it exists for only about 10^{-13} sec.

In molecular terms, these transition states reflect changes in bond lengths and bond angles as well as the formation and breakage of bonds. The energy required to achieve a transition state is denoted as the activation energy which may also be considered as the difference in energy between the energy of the transition state and the energy of the reactants. According to Pauling's hypothesis, an enzyme preferentially binds the transition state of a reaction, thereby stabilizing it relative to the substrate and products and reducing the activation energy of the reaction, thus increasing the reaction rate. For example, aspartic proteinases are enzymes which are known to catalyze the hydrolysis of peptide linkages within a protein molecule.

By extending this explanation, Pauling also predicted that stable analogs of a transition state would bind tightly to an enzyme. It has been suggested that the term "transition state analog" might be used to describe an inhibitor of this kind (1).

Pauling's prediction has become the basis for the now well established approach to enzyme inhibitor design. The strategy for designing enzyme inhibitors has suggested a strategy for preparing catalytic antibodies whereby antigens are designed based upon mechanistic principles so that antibodies raised in response to such antigens will catalyze a chemical reaction by carrying out the reaction mechanism implicit in the design of the antigen. This strategy has been attempted a number of times.

For example, a transition-state analog mimicking an intramolecular 6-member ring cyclization transition state was used to elicit a monoclonal antibody which acted as a stereospecific, enzyme-like catalyst (2). Specifically, the monoclonal antibody so elicited accelerated, by about a factor of 170, the formation of a

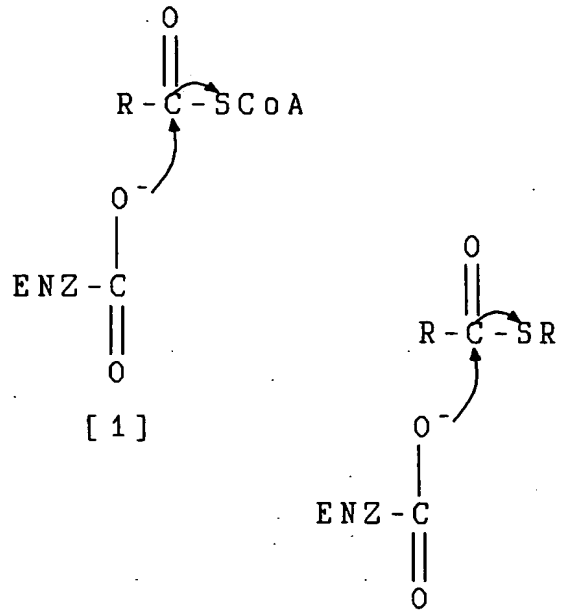
single enantiomer of a δ-lactone from the corresponding racemic δ-hydroxyester.

Similarly, monoaryl phosphonate esters, designated as analogs of the transition state in the hydrolysis of carboxylic esters, were synthesized and used as haptens to elicit specific monoclonal antibodies capable of catalyzing the hydrolysis of carboxylic esters (3). Certain of the antibodies elicited were reportedly found to be catalytic and selective for the hydrolysis of particular aryl esters.

Phosphonamidates or phosphonate analog-ligands having conformations that substantially correspond to the conformation of a hydrolytic transition state of an amide or ester ligand and which have been used to produce antibodies are described in U.S. Patent 4,659,567 to Tramontano et al. (Tramontano). Antibodies so produced purportedly include a paratope that binds to and stabilizes the tetrahedral carbon atom of the amide or ester hydrolysis transition state of the ligand to hydrolyze the ligand at a predetermined site.

Analog-ligands which can be used to produce antibody catalysts for the hydrolysis of esters and amides are also described in European Patent Application 0,251,093 of Kollmorgen Corp. (Kollmorgen).

In enzyme catalysis, groups on both the substrate and the enzyme which are not involved in the chemical mechanism of bond making and breaking make an important contribution to catalysis. This is illustrated by examining the action of the enzyme succinyl-CoA acetoacetate transferase, shown below, which involves nucleophilic attack of the enzyme's glutamate carboxyl on the thioester succinyl-CoA [1] to give an anhydride intermediate. The enzyme forms anhydride intermediates from "non-specific" substrates [2], as well.



[1]

[2]

Even though the chemical reactivities of the two substrates, [1] and [2], are similar, e.g. towards alkaline hydrolysis, the enzyme reaction proceeds up to 3 X 10^{12} fold faster with the so called specific substrate [1](4). The non-reacting part of the substrate, i.e., the CoA residue, lowers the activation energy by 72 KJmol^{-1} compared with [2].

It has also been explicitly noted that to obtain catalysis, the Gibbs free energy of the enzyme-substrate and enzyme-product complex must be increased so that the transition state can be reached easily (5). This represents destabilization of the enzyme-substrate complex which can occur by physical strain, desolvation and other mechanisms.

Thus, the haptens disclosed in Tramontano do not provide the correct architecture to elicit antibodies that are capable of catalyzing the cleavage of a predetermined peptide sequence in a native protein. These haptens do not provide the correct side-chain groups for production of antibodies that can react with predetermined sites on a protein and cause selective

proteolysis in a sequence specific manner. Furthermore, these haptens do not incorporate amino acid side-chain sub-sites on either side of the transition state analog. Without these sub-sites, the haptens cannot provide for the elicitation of catalytic antibodies capable of recognizing a specific amino acid sequence and selectively proteolyzing a peptide linkage within that sequence. To date, no general method for eliciting antibody peptidases has been developed.

OBJECTS, FEATURES AND ADVANTAGES OF THE INVENTION

It is an object of the invention to provide a rational design approach for designing haptens which are capable of molecular recognition and hydrolytic rate enhancement.

It is also an object of the invention to provide haptens which include an array of atoms which mimic the transition state in the cleavage or formation of a peptide bond and/or which mimic a distorted group state.

It is a further object of the invention to provide haptens which mimic the native conformation of biomolecules and which have complimentarity with biomolecules.

It is another object of the invention to provide catalytic antibodies which are capable of catalyzing the cleavage or formation of a peptide bond.

It is yet another object of the invention to provide catalytic antibodies capable of recognizing a specific amino acid sequence in a molecule containing numerous amino acids.

It is a further object of the invention to provide catalytic antibodies which are capable of catalyzing the cleavage or formation of a peptide bond within a specific amino acid sequence of a molecule.

It is yet another object of the invention to provide a method for catalyzing the cleavage or formation of a peptide bond in a molecule.

It is a further object of the invention to provide a method for catalyzing the cleavage or formation of a specific peptide bond within a specific amino acid sequence of a molecule containing numerous peptide residues joined by peptide bonds.

These and other features and advantages of the invention will become readily apparent from the ensuing detailed description, and the novel features will be particularly pointed out in the appended claims.

SUMMARY OF THE INVENTION

The invention is broadly directed to antigens capable of eliciting through immunogenic methods catalytic antibodies which can catalyze the cleavage or formation of an amide, peptide, ester or glycosidic bond in a molecule. The invention is directed to antigens capable of eliciting through immunogenic methods catalytic antibodies which can catalyze the selective cleavage or formation of a predetermined amide bond in a native polypeptide sequence. In general, the antigens can be a hapten or an immunogen comprising a hapten coupled (linked, conjugated) to a carrier molecule via a suitable coupling moiety. The haptens include structural elements which are designed to (i) mimic one or more high energy intermediates or transition states in the cleavage or formation of the amide, ester or glycosidic bond and/or (ii) mimic one or more high energy conformations of the amide, ester or glycosidic bond to be cleaved.

The haptens according to the invention provide the correct side chain groups for production of antibodies that can react with predetermined sites on a protein and can catalyze selective proteolysis in a sequence specific manner. The haptens further incorporate amino acid side-chain sub-sites surrounding the amide bond analog. These sub-sites provide for the elicitation of catalytic antibodies capable of recognizing a specific amino acid sequence and

selectively proteolyzing a peptide linkage within that sequence.

Such catalytic antibodies are elicited with the haptens of the present invention. For example, a hapten according to the invention, shown below,

sub-sites sub-sites

A_{aa} -- B_{aa} --[CD]-- E_{aa} -- F_{aa}

dipeptide analog

incorporates not only the dipeptide analog [CD] but also sub-site amino acid residues A, B, E, F. These subsite amino acid residues can be part of a cyclic structure as well as a linear structure. The optimum number of sub-site residues is determined by the size of the antibody combining site. It is likely that the only essential criterion for effective binding of antibody to a peptide is that complementarity between the antigen combining site of the antibody and the molecular surface of the binding peptide is maintained with regard to both shape and charge.

The haptens according to the invention are designed in such a way such that antibodies raised against these haptens can selectively stabilize one or any of the high energy intermediates or transition states in the cleavage or formation of an amide, peptide, ester or glycosidic bond and/or destabilize the bound ground state. These haptens fall into three general classes: one, those in which the hybridization of the atom corresponding to the carbonyl carbon of the scissile bond of the amide or ester bond is converted from sp^2 to sp^3 hybridization; two, those in which any of the atoms corresponding to the amide, ester or glycosidic bond is replaced by a different atom; and three, those in which the atoms corresponding to the amide, ester or glycosidic bond are part of a monocyclic or bicyclic system.

Peptide sequences containing dipeptide analogs, according to the invention, at the bond that is required

to be hydrolyzed by the catalytic antibodies of the present invention define a sequence that the catalytic antibody will hydrolyze in a native protein. The binding energy of the antibody is distributed in such a way as to allow both sequence specific recognition and chemical reactivity with the native protein or peptide of interest.

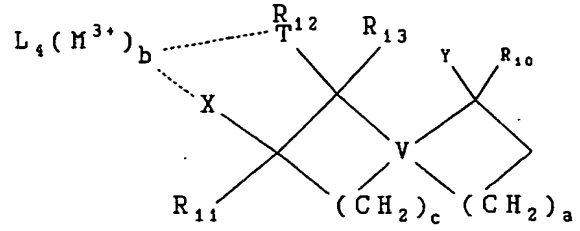
It has been reported that it is not necessary to prepare peptides longer than eight amino-acid residues (octapeptides) to demonstrate all continuous epitopes (6). It has also been demonstrated that antibodies bind to peptides in a reproducible manner (7). It has also been established that optical isomerism of the amino acids used has a powerful influence on the strength and specificity of antibody binding by dipeptides. Consequently, the importance of L and D amino acid residues in the immunizing antigen will have a profound effect on the chirality of the antibody combining site generated. In generating catalytic antibodies according to the invention with predetermined specificity for particular sequential (continuous) or assembled epitopes in a native protein, the relationship between measurable properties of a protein and its immunogenic sites are important (8). With the ready availability of protein sequences, the most widely used algorithm is based on the likelihood of finding a sequential epitope at the site of a local maximum in the hydrophilicity profile (9). Surface accessibility profiles (10) and protein flexibility (11) also provide information on the antigen sites in a native protein sequence. With knowledge of these sites and the importance of these epitopes in receptor mediated interactions or other disease associated mechanisms, peptide haptens having dipeptide analogs within these important "bioactive" epitopes can be designed in accordance with the invention. The catalytic antibodies elicited with these haptens can then be utilized, for

example, to digest epitopes on viral proteins or tumor derived growth factors or other peptides involved in life-threatening situations (e.g., tumor necrosis factor in bacterial sepsis, etc.).

Thus, the haptens of the invention are distinguished from prior analog-ligands in that they have been rationally designed from knowledge of mechanistic features of enzyme catalysis and provide suitable templates for generating antibody combining sites endowed with catalytic properties. Consequently, they incorporate all the necessary features to provide for antibodies capable of molecular recognition and catalytic function.

Accordingly, the invention is a method for catalyzing the cleavage or formation of a specific peptide bond within a molecule. The method comprises contacting the molecule with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of the peptide bond under conditions suitable for the cleavage or formation to take place; the monoclonal antibody having been prepared by a process comprising the steps of: selecting the specific peptide bond to be cleaved or formed; selecting an antigen comprising an analog of the peptide bond to be cleaved or formed, and also comprising moieties surrounding the analog of the peptide bond, which moieties substantially correspond to some or all of the moieties surrounding the peptide bond to be cleaved or formed; exposing cells capable of producing antibodies to the antigen and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the cleavage or formation of the peptide bond.

In one aspect, the invention is directed to a hapten of formula XVIII



XVIII

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 0 to 10;

R_{10} and R_{11} may be the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

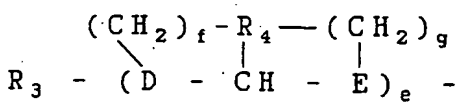
L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N^+ with any negatively-charged counterion, or C;

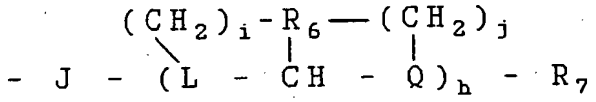
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=0 or C=S;

J is NH, O, S, CH_2, CF_2, C=0 or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

and wherein

one or more of R_{10}, R_{11}, R_4 and R_6 are unbound or bound to one or more of said remaining substituents R_{10}, R_{11}, R_4 and R_6 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$,
$-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$,
$-(CH_2)_u-NH-(CH_2)_v-$, and $-(CH_2)_u-$phenyl$-(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

The haptens of formula XVIII may be incorporated into a peptide sequences at the bond that is required to be hydrolyzed by the catalytic antibodies of the present invention. The subsite residues of the peptide sequence result in the elicitation of antibodies

capable of recognizing a specific amino acid sequence and cleaving the peptide bond therein.

The foregoing haptens may be used as antigens for in vitro elicitation of catalytic antibodies. However, for purposes of in vivo elicitation, the haptens must be coupled to a suitable carrier molecule in order to obtain an immunogen suitable for immunization. Therefore, the invention is also directed to immunogens capable of eliciting catalytic antibodies. Such immunogens comprise a hapten as hereinbeforedescribed coupled to a carrier molecule by a suitable coupling moiety.

In another aspect the invention is directed to catalytic antibodies which are elicited by antigens comprising the haptens of the invention as described above. Similarly, the invention is also directed to catalytic antibodies which can catalyze a chemical reaction of interest and which are elicited through in vitro or in vivo techniques by antigens comprising haptens according to the invention as described above, wherein the antibodies have been prepared by exposing cells capable of producing antibodies to the antigens and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby producing a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the chemical reaction of interest.

Alternatively, cells capable of producing catalytic antibodies can be stimulated to grow in culture and, therefore, can be immortalized using methodologies well known in the art. For example, lymphocytes can be so stimulated using a virus, a chemical agent or a nucleic acid (e.g., an oncogene).

In still another aspect, the invention is directed to a method for producing catalytic antibodies

which can catalyze a chemical reaction of interest and which are elicited through in vitro or in vivo techniques by antigens comprising the haptens according to the invention as described above. The method comprises exposing cells capable of producing antibodies to the antigens and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the chemical reaction of interest.

The invention is also directed to a method for catalyzing the cleavage or formation of a peptide bond in a molecule. The method comprises contacting the molecule with an effective amount of a catalytic antibody which has been elicited by antigens comprising haptens according to the invention.

In another aspect, the invention is directed to a method for catalyzing the cleavage or formation of a specific amide bond within a specific amino acid sequence of a molecule containing numerous amino acids joined by amide bonds. The method comprises contacting the molecule with an effective amount of a catalytic antibody which has been elicited by antigens comprising haptens according to the invention. The haptens have complimentarity with the specific amino acid sequence.

In a particularly preferred embodiment, the invention is directed to a spiro [4.4] nonane-containing dipeptide analog useful as a hapten.

As noted earlier, the catalytic antibodies elicited by antigens comprising haptens according to the invention can be used, for example, to digest epitopes on viral proteins or tumor-derived growth factors on other peptides involved in health- or life- threatening situations.

With the foregoing and other features and advantages of the invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference to the following detailed description of the invention, the accompanying drawings, and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A depicts torsionally-distorted glycyl-proline.

Fig. 1B depicts a spiro analog of glycyl-glycine or glycyl-proline.

Fig. 1C depicts torsionally distorted glycyl-glycine.

Fig. 2 is the reaction sequence for the synthesis of *trans*-*tert*-butyldimethylsilyloxyspiro[4.4]nonane-6-ene and *cis*-*tert*-butyldimethylsilyloxyspiro[4.4]nonane-6-ene.

Fig. 3 is the reaction sequence for the synthesis of methyl-*trans*-6-hydroxyspiro[4.4]nonane-*trans*-1-carboxylate and methyl-*trans*-6-hydroxyspiro[4.4]nonane-*cis*-1-carboxylate.

Fig. 4 is the reaction sequence for the synthesis of 7-oxa-6-oxotricyclo[6.3.0.$0^{1,5}$] undecane.

Fig. 5 is the reaction sequence for *N*-(D-tyrosyl)-*trans*-7-amino-*trans*-6-hydroxyspiro[4.4]nonane-1-carboxyl-D-phenylalanine.

DETAILED DESCRIPTION OF THE INVENTION

Broadly, the invention relates to antigens which are capable of eliciting through immunogenic methods antibodies which can catalyze the cleavage or formation of a peptide linkage or the cleavage or formation of an ester in a molecule. These antigens comprise a hapten or a hapten and a suitable carrier molecule. Because the antibodies so elicited can catalyze a chemical reaction, they are defined as "catalytic antibodies." Catalytic antibodies are

identified and described in U.S. Patent No. 4,888,281, referred to above in the "Background of the Invention."

During the course of a chemical reaction, the reactants undergo one or more transitions through structures which are energetically less favorable than either the reactant or product. In molecular terms, these transition states (or intermediate structures) reflect changes in bond lengths and bond angles as well as formation and breakage of bonds. The energy required to achieve a transition state is denoted as the activation energy, which may also be considered as the difference in energy between the energy of the transition state and the energy of the reactants.

Catalysts increase chemical reaction rates by lowering the activation energy of a reaction. Antibodies elicited to a hapten or immunogen of the invention, which antigens are chosen because, inter alia, they resemble the presumed transition state structure, a strained ground state structure, or both, can catalyze reactions. The antibody thus produced should stabilize the energy of the transition state relative to reactants and products and/or destabilize the energy of the bound substrate relative to unbound substrate. This approach has been successfully demonstrated in the generation of several catalytic monoclonal antibodies.

Catalytic antibodies elicited with haptens according to the invention are "site specific" in that they are deliberately designed only to catalyze cleavage of peptide bonds having certain structural conformations at specific sites in a protein molecule. Likewise, these catalytic antibodies are designed only to catalyze the formation of peptide linkages from the N- and C-termini of amino acids having certain structural conformations at those termini. Therefore, haptens according to the invention may be used to elicit a site specific catalytic antibody capable of cleaving peptide

bonds at specific sites in a protein molecule to produce two or more cleaved protein strands. The same catalytic antibody can then catalyze the formation of peptide bonds wherein those cleaved strands having the right structural conformation are joined.

Thus, the haptens of the invention are designed to mimic the transition-states or strained ground states or both for a variety of chemical reactions. Preferably, though not exclusively, the reactions are the cleavage or formation of a peptide bond. Certain haptens of the invention may also be able to mimic the transition states of other non-peptide types of chemical reactions.

In an embodiment of the invention, a method is provided for catalyzing the cleavage or formation of a specific peptide bond within a specific amino acid sequence contained in a molecule. The molecule is contacted with an amount of a monoclonal antibody effective to catalyze the cleavage or formation of the peptide bond under conditions suitable for the cleavage or formation to take place. The monoclonal antibody is elicited using an antigen comprising a hapten of the invention.

The term "amide bond" refers to a simple amide bond (e.g., an amide bond in a side chain of a naturally occurring amino acid) or an amide bond which joins two adjacent amino acid residues, i.e., a peptide bond. The term "peptide" includes dipeptides and polypeptides.

The term "analog of an amide bond" as used herein is defined as a normal amide bond (-CO-NH-) in which one or more moieties in the normal amide bond are replaced by one or more different moieties similar in charge and/or size to the normal moieties replaced. "Moiety" is defined as a radical (e.g., an atom, CH_3, C_6H_5, OH, NH_2, etc.). For example, in one embodiment of the invention, an analog of an amide bond is $-CO-CF_2$ wherein the normal NH moiety is replaced by the CF_2 moiety.

In its broadest sense, the term "antigen" is defined as a molecule which induces the formation of an antibody. As used herein, the term "antigen" means a molecule which is inherently immunogenic, a hapten according to the invention or an immunogen which comprises a hapten according to the invention coupled to a carrier molecule by a suitable coupling moiety. Carrier molecules include, for example, keyhole limpet hemocyanin (KLH), thyroglobulin, chicken immunoglobulin, ovalbumin, bovine serum albumin (BSA), T-helper peptides, etc. "Coupling moiety" as used herein refers to biotechnological cross-linking reagents well known in the art (e.g., commercially available from Pierce, Rockford, Illinois) and include, for example, Trout's reagent, dissuccinyl suberate, etc.

The term "transition state analog" as used herein refers to an array of atoms which is designed to approximate or "mimic" the configuration of an amide bond or an ester bond as such bonds exist in a hydrolytic transition state.

The term "strained ground state" as used herein refers to an array of atoms that is designed to approximate or "mimic" one or more high energy conformations of an amide or ester bond.

The term "dipeptide analog" as used herein refers to a structure which comprises a transition state analog or strained ground state analog or elements of both having side chains of two amino acids which are in positions analogous to those of the dipeptide being mimicked. In other words, in a dipeptide analog, the normal amide bond (i.e., -CO-NH-) between the two amino acids has been replaced by an array of atoms as defined above. Additional amino acid residues may be incorporated to surround the dipeptide analog to form a polypeptide. Thus, the dipeptide analog replaces the peptide bond "targeted" for cleavage in the substrate molecule. In one embodiment of the invention, the

moieties surrounding the dipeptide analog contain peptide bond linkages which can be altered such that the naturally occurring C=O group is replaced by NH, O, S, CH_2, CF_2 or C=S and/or the naturally occurring NH group is replaced by O, S, CH_2, CF_2, C=O or C=S. For example, the moieties can be retropeptides in which the C=O and NH groups of the amide bonds are interchanged.

The terms "some or all" refer to a portion of the target molecule including at least the bond to be cleaved or all of the target molecule. For example, in an embodiment of the invention, haptens designed for the purpose of eliciting antibodies to catalyze the cleavage of a specific peptide bond in a protein molecule comprising a polypeptide of many amino acid residues, the dipeptide analog corresponding to the target peptide bond need only be surrounded by not more than about eight amino acid residues. However, if the target molecule is a relatively short peptide, it is advantageous to surround the peptide bond analog with all the amino acid residues of the target molecule. Of course, one of ordinary skill in the art will realize that the desired specificity, the nature of the target molecule and other factors will dictate the ideal number of amino acid residues needed to surround the dipeptide analog.

The term "substantially corresponds" refers to moieties which are similar in charge and/or size to moieties in the amide bond analog, dipeptide analog or naturally occurring amino acid side chain analog. Preferably, the moieties are identical in size and charge, although such identity is not necessary for the hapten of the invention.

The term "hapten" as used herein is defined as a molecule which can act as an epitope. Haptens according to the invention incorporate a dipeptide analog according to the invention.

Physiologically acceptable salts include salts of mineral acids, for example, hydrochloric acid,

sulfuric acid, nitric acid and the like, salts of monobasic carboxylic acids such as, for example, acetic acid, propionic acid and the like, salts of dibasic carboxylic acids such as, for example, maleic acid, fumaric acid and the like, and salts of tribasic carboxylic acids such as, for example, citric acid and the like.

The term "naturally occurring amino acid" as used herein includes the twenty essential alpha-amino acids and other alpha-amino acids which may or may not be found in proteins. These amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, 4-hydroxyproline, 5-hydroxylysine, epsilon-N-methyllysine, 3-methylhistidine, beta-alanine, gamma-aminobutyric acid, homocysteine, homoserine, citrulline, ornithine, canavanine, djenkolic acid and beta-cyanoalanine. An amino acid consists of a carbon atom to which is bonded an amino group, a carboxyl group, a hydrogen atom and a distinctive group referred to as a "side chain". In proline, the side chain is also bonded to the nitrogen atom of the amino group, thus forming a cyclic structure.

Accordingly, the above-described haptens include a side chain of a naturally occurring amino acid as well as an "analog of said side chain." The term "analog of said side chain" as used herein is defined as a side chain of a naturally occurring amino acid in which one or more moieties of the naturally occurring side chain is replaced by one or more different moieties which substantially corresponds to the naturally occurring moiety. Those side chains containing a hydroxy group can be glycosylated, phosphorylated, sulphonylated or protected by a hydroxy protecting group. The hydroxy group of any of the side chains may be protected by any

number of suitable hydroxy protecting groups well known in the art. These include, for example, a tertiary butyl group.

The term "terminal amino protecting group" means any group capable of protecting the terminal amino moiety of a peptide or amino acid. Therefore, terminal amino protecting groups include acetyl, succinyl, biphenylcarbonyl, benzoyl, t-butyloxycarbonyl, carbobenzyloxy, tosyl, dansyl, isovaleryl, phthalyl, 1-adamantanesulphonyl, acetimido, benzimido, amidino, carbamyl and the functional equivalents thereof.

The term "terminal carboxyl protecting group" means any group capable of protecting the terminal carboxyl moiety of a peptide or amino acid. Terminal carboxyl protecting groups include (C_1-C_9)alkyl, phenyl, substituted methyl esters such as methoxymethyl and phenacyl esters, 2-substituted ethyl esters such as cyclohexyl and allyl, substituted benzyl esters such as *para*-methoxybenzyl and *para*-bromobenzyl, amides such as piperidinyl and hydrazide and functional equivalents thereof.

The value of 2 for variables f, g, i and j provides for the possibility of a proline ring structure. However, it will be understood that when any of e, f, g, i or j is zero, there is no proline ring structure at the respective sites of those variables in the hapten.

L_4 is a ligand in formula XVIII, and preferably L_4 is $4H_2O$, $4NH_3$, 2 ethylene-diamine or triethylenetetramine.

Haptens according to the invention contain one or more asymmetric centers and therefore exist in enantiomeric and/or diastereomeric forms. In general, the corresponding haptens according to the invention are obtained in the form of racemates or mixtures of diastereomers. If desired, techniques well known in the art for the separation of the mixtures into stereochemically homogeneous constituents may be used.

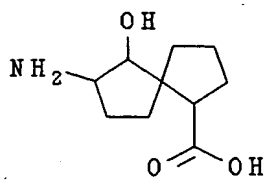
Preparation of the optical isomers in a pure state is also possible by using stereochemically homogeneous starting materials.

One of ordinary skill in the art will realize that arrays containing carbonyl adjacent to difluoromethylene or fluoromethine and boron will assume a tehrahedral like configuration upon reaction with water in an aqueous environment.

Preferred haptens of formula XVIII include those haptens wherein V is carbon; X is NH_2; Y is COR_5; a and c are both 2; and R_{10}, R_{11}, R_{12} and R_{13} are all hydrogen.

Preferably the amino moiety of X is bound to the carboxyl terminus of an amino acid, e.g. tyrosine, and the carboxyl moiety of Y is bound to the amino terminus of an amino acid, e.g. phenylalanine, by covalent bonds or linker moieties as defined above.

An especially preferred hapten of the present invention has the formula:



One application of catalytic antibodies having site-specific proteolysis capabilities is in the immunotherapy of viral infection. Viruses utilize their external coat proteins to attach to cellular receptors nd invade the cell after attachment. For example, human immunodeficiency virus (HIV) uses a portion of the gp120 protein at its surface to attach to CD4 receptors on lymphocytes. The sequence for this cell attachment has been mapped to a region on the viral protein. With this information, antibodies can be generated by the methodology described in this invention to bind to this peptide sequence and cleave it in a site-specific manner.

However, such antibodies preferably bind to the "native" sequence in the protein as opposed to a linear sequence (which would occur in a denatured protein). Thus, the antigenic determinants or epitopes in the "native" protein are often conformational (i.e., three-dimensional) rather than random linear arrangements. Here again, knowledge of epitopes on the protein is important in the design of antibodies having paratopes that can induce modifications of such epitopes.

Therefore, haptens according to the invention are designed to have the same structural features of the epitopes, rather than random conformations. These structural features can be adopted by simple linear peptides, the lowest energy conformer being the preferred structure in solution. Secondary structural features may be introduced by cross-linking of amino-acid side-chains or the use of β-turn mimetics. Conformationally constrained haptens incorporated structures which are compatible with the epitope in the native protein may be essential for inducing the correct motif within the tertiary structure of the catalytic antibody hyper-variable binding region. The advantages of conformationally constrained haptens are that they mimic the native structure in the protein and tend to mimic regions of the protein which are susceptible to cleavage. Accordingly, in the structural formula XVIII shown above for the haptens of the invention, the substituents R_{10}, R_{11}, X and Y may be bound to one or more of the remaining substituents R_{10}, R_{11}, X and Y by a covalent bond or a linker moiety as defined above.

The haptens of the invention can take on a configuration mimicking that of the native ß-turn or "hairpin" configuration of proteins by the formation of disulphide bridges between sulfur containing amino acid side chains which are incorporated into the hapten. Formation of disulphide bridges also promotes hydrogen

bonding interactions. Disulphide bridge formation can be achieved by chemical methodology well known in the art.

The utility of the antigens of this invention coupled with appropriate screening procedures and reiterative thermodynamic perturbation studies of transition-state structures and free-energies of interaction with catalytic groups provide a methodology for production of catalytic antibodies by a rational design approach.

The invention also is directed to catalytic antibodies which are elicited by antigens comprising haptens according to the invention. These antibodies may be monoclonal or polyclonal but are preferably monoclonal and may be in the form of purified immunoglobulins (IgG, IgM, IgA, IgD or IgE) or antibody fragments, such as Fab, $F(ab')_2$, F_v, etc., of immunoglobulins.

A catalytic antibody in accordance with the invention is a substance which is capable of changing the rate of a chemical reaction, all other conditions (e.g., temperature, reactant/substrate concentration, etc.) being the same and which does not enter into the chemical reaction and therefore is not consumed in the reaction. It is also a substance which exhibits the capability of converting multiple moles of reactant/substrate per mole of catalytic antibody; which, from a mechanistic viewpoint, binds the reactant/substrate, effects the accelerated conversion of the reactant/substrate to the product and then releases the product; and which changes the rate of the chemical reaction without shifting the position of the equilibrium. The aforementioned definitions are characteristics of ideal catalysts. However, in practice, even the best of catalysts become poisoned or deactivated by contamination in the reaction system or as a result of chemical or physical destruction during the reaction process. For reasons well known in the art, the true operation of a catalyst may be obscured

by components of the reaction system or by the condition of the reaction environment.

The art has adopted certain working definitions to express catalytic activity. These expressions are [1] k_{cat}, or "turnover" and [2] k_{cat}/k_{uncat}, the "rate enhancement factor". Turnover indicates the number of molecules of reactant/substrate which can be converted to product per molecule of catalytic antibody per unit time. For example, if a molecule exhibits a turnover of 10^3 molecules of substrate per minute and the molecule maintains its catalytic activity for 24 hours at room temperature and at its optimal pH, each molecule of catalyst would then make a total of 1.4×10^6 conversions, indicating its catalytic behavior. This total conversion is to be distinguished from the total conversion in a stoichiometric reaction, which will never exceed 1.0, no matter how long the reaction is carried out. The rate enhancement factor is a dimensionless number which expresses the rate of reaction in the presence of catalyst to the rate of reaction in the absence of catalyst, all other reaction conditions (e.g., reactant concentration, temperature, etc.) being equal.

Catalytic antibodies according to the invention may be elicited through both in vitro and in vivo techniques. The term "elicited" as used herein means elicitation of catalytic antibodies by antigens according to the invention through both in vitro and in vivo techniques. However, the skilled artisan will readily appreciate that when in vitro elicitation is involved, the haptens according to the invention, by themselves, may be used to elicit the catalytic antibodies. However, when elicitation is achieved through in vivo techniques, it is understood that immunogens comprising haptens complexed to a suitable carrier molecule are used to elicit the catalytic antibodies. Another aspect of the invention is directed to a method for producing antibodies which can catalyze a chemical reaction of

interest and which are elicited through in vitro or in vivo techniques by an antigen. The antigen comprises a hapten according to the invention. The haptens are designed to elicit the appropriate hypervariable binding region in an antibody molecule to express intrinsic binding energy for the transition- state of a chemical reaction, particularly a hydrolytic reaction. Arrangement of amino-acid side chains generated in the combining-site will be appropriate for performing chemical modification of an epitope of interest. Additional improvements in catalytic efficiency can be achieved by site-directed mutagenesis.

Broadly, the method comprises exposing cells capable of producing antibodies to the antigen and thereby generating antibody producing cells; hybridizing the antibody producing cells with myeloma cells and thereby producing a plurality of hybridoma cells each producing monoclonal antibodies; and screening the plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes the chemical reaction of interest. The monoclonal antibody so identified may then be replicated, again by either in vivo or in vitro techniques, to obtain a quantity sufficient to catalyze the chemical reaction of interest.

The detection of antibodies with the desired catalytic activity and specificity is achieved by screening the hybridomas once they have been elicited. For example, screening may be achieved by high performance liquid chromatography (HPLC) or spectrophotometric methods (ELISA). Catalytic monoclonal antibodies are elicited "in vivo" by modification of the technique disclosed by Koprowski et al. in U.S. Patent No. 4,196,265, issued April 1, 1980, which is hereby incorporated by reference. The details of that process are known in the art. A series of monoclonal antibodies directed to a specific molecule are prepared under suitable conditions. This involves first

immunizing BALB/C mice with an appropriate antigen. The antigen comprises a hapten according to the invention bound to a peptide or other carrier molecule.

Antibody-producing lymphocytes are then removed from the spleens of the immunized mice and hybridized with myeloma cells such as SP2/0 cells to produce hybridoma cells. These hybridoma cells are then plated in the wells of microliter plates. The series of monoclonal antibodies being produced by the hybridoma cells is screened under appropriate conditions to identify monoclonal antibodies which catalyze the desired reaction under appropriate conditions. Alternatively, the medium may be tested for antibodies that bind to the immunogen and the hybridomas producing these antibodies then expanded in tissue culture or grown in vivo. Screening may be conveniently accomplished by treating a standardized solution of the reactant with an aliquot of medium withdrawn from a microliter well and measuring the presence of the desired product by conventional instrumental methods. This measurement may be readily conducted, for example by spectrophotometric methods or by gas-liquid or high pressure liquid chromatography. By comparison with standardized samples of the desired product or reactant, rates of reaction may be quantified. In this manner, wells containing hybridoma cells producing catalytic monoclonal antibodies are identified. The selected hybridoma cells are then cultured to yield colonies.

These colonies may be further propagated in vitro or in vivo systems. In the latter case, mice such as syngeneic BALB/c mice are inoculated intraperitoneally with the selected hybridoma cells and produce tumors, generally within two or three weeks. These tumors are accompanied by the production of ascites fluid which contains the desired monoclonal antibodies. The monoclonal antibodies are then separately recovered from the ascites fluid by conventional methods such as

ultrafiltration, ultracentrifugation, dialysis and immunoffinity chromatography.

The invention is also a method for catalyzing the cleavage or formation of an amide (peptide) bond in a molecule. The molecule can be a natural or synthetic peptide or protein. Target molecules include biomolecules which are defined as any molecule which affects a biological system in vivo or in vitro. Biomolecules may be synthesized by cells or chemically synthesized. Examples of biomolecules include proteins, glycoproteins, peptides, steroids, and maleic acid. Also included are synthetic organic analogs of peptides, steroids, maleic acid, etc. Pharmaceutically active compounds such as theophylline, caproin, cyclosporin, etc., are also considered to be biomolecules. The method comprises contacting a molecule containing one or more amide (peptide) or ester bonds with an effective amount of a catalytic antibody which has been elicited by an antigen comprising a hapten of the invention.

In accordance with the invention, the separately recovered monoclonal antibodies are contacted with a molecule under suitable conditions permitting the formation of a complex between the monoclonal antibody and the molecule. In general, the concentration of the catalytic antibodies used is less than the equivalent concentration of the target molecule and may be in the picomolar range. The antibodies should function under normal physiologic conditions in vivo. The skilled artisan will appreciate that the conditions suitable for complex formation may vary depending on the particular molecule and monoclonal antibody under consideration.

Accordingly, the methods of this invention may be practiced under a variety of reaction conditions, in vivo and in vitro, as long as the monoclonal antibodies are not prevented from complexing with the molecules or otherwise rendered inactive. More specifically, suitable

conditions for complex formation encompass solution phase and emulsion reaction systems including a protic solvent, preferably water, maintained at a pH value between about 6.0 and about 9.0, preferably between about 6.0 and about 7.5 and at a temperature from about 4°C to about 50°C, preferably from about 20°C to about 45°C. The ionic strength = $1/2\ \Sigma\ c_i z_i^2$, where c is the concentration and z is the electronic charge of an ionic solute, should be maintained at a value below about 2.0 moles/liter, preferably between 0.1 and 1.5 moles/liter. The method of this invention may be carried out at reduced or elevated pressure, but preferably is practiced at ambient pressure. In addition to solution phase and emulsion reaction systems, suitable conditions also include the use of support materials to which the monoclonal antibody is attached. Such support materials are well-known to those of ordinary skill in the art as are methods for attaching monoclonal antibodies to them.

Catalytic antibodies elicited with the antigens of the invention may be useful in the treatment of autoimmune disease, cancer and thrombolytic disease. The catalytic antibodies may also be useful for treatment of cardiovascular disease eliminating high density lipoproteins and for the detoxification of bacterial endotoxins. Vaccines comprising synthetic peptides optionally linked to carrier proteins, for example, against foot and mouth disease (FMD) and E. coli enterotoxin, have been proven efficacious in recent years.

Oligopeptides having variable lengths with sequences from the receptor-binding regions of viruses which employ a specific cellular receptor for penetration of the host cell and having a transition state analog dipeptide isostere in a critical region of the sequence induce on immunization, optionally after coupling to a suitable carrier protein, catalytic antibodies that cleave the viral coat protein and prevent

virus penetrating the cell. The dimensional structure of Rhino 14 and Polio 1 virus particles has been charted by X-ray scattering. Regions have been identified which are binding sites to cellular receptors. The region of the human immunodeficiency virus type 1 (HIV I) critical for interaction with the CD4 receptor on T-lymphocytes has been located and mapped to sequences in the gp120 goat protein. Thus, in one embodiment of the invention, oligopeptides are used which contain partial sequences from the envelope proteins of viruses critical for host cell attachment and a transition-state dipeptide isostere selected from the haptens according to the invention. The resultant peptide analogs are used to induce catalytic antibodies that inactivate viruses by proteolysing segments (epitopes) of the viral coat protein critical for infectivity. Preferably, oligopeptides are used having sequences from the receptor binding region of retroviruses e.g., HIV I, HIVII and picorna viruses, e.g., Rhino 14, viral polypeptides, inflammatory proteins, anaphylactic proteins, lymphokines, cytokines and other polypeptide mediators of host infection or toxic syndromes.

It will be understood by the skilled artisan that the following syntheses may be modified to provide other haptens of the invention.

The novel spiro[4.4]nonane-containing dipeptide analog 7-*trans*-amino-6-*trans*-hydroxyspiro-[4.4]nonane-1-carboxylic acid, as a racemic mixture, has been synthesized and incorporated into a longer peptide sequence. This dipeptide analogue mimics both torsionally-strained glycyl-proline and glycyl-glycine as shown in Figure 1; in addition, the peptide bond has been replaced by a hydroxyethylene group, an effective transition state analogue.

A key step in the synthesis of the spiro[4.4]nonane-containing dipeptide analogue was the introduction of an amino functionality into the

corresponding hydroxyester **12** via an intramolecular acyl-nitrene insertion reaction. Thus a stereoisomer of this material, **12**, was first synthesized as is outlined in Fig. 2 and 3. The monoreduction of racemic spiro[4.4]nonane-1,6-dione **1** with lithium tri-*tert*-butoxyaluminohydride at -30 °C yielded, as described by Carruthers and Orridge, predominantly the *cis*-isomer of 6-hydroxyspiro[4.4]nonane-1-one **2**. (As outlined in Fig. 4 below, upon further elaboration, the *cis*-hydroxyketone yielded the lactone **13**, which resisted hydrolytic and methanolytic opening, and hence was unusable in the insertion reaction.) Platinum oxide catalyzed reduction of the dione **1**, which had been reported by Hardegger et al. [J. Am. Chem. Soc. (1959) 81:2727-2729)] to yield predominantly the *trans*-isomer again yielded the *cis*-isomer as the major product. Finally, by the slow addition of lithium tri-*tert*-butoxyaluminohydride to the dione **1** at 0 °C, the *trans*-isomer of **2** was obtained as the major product (approx. 70% by ^{1}H NMR). This isomeric mixture of **2** was protected to give the *tert*-butyldimethylsilyl derivative **3**. Homologation to the alkenes **4** and **5** was accomplished with methyltriphenylphosphonium bromide in sodium dimsylate. The isomeric alkenes **4** and **5** separated during chromatography on silica gel. (The *tert*-butyldimethylsilyl derivative **3** was unreactive with 2-(trimethylsilyl)-2-lithio-1,3-dithiane, and methoxymethylenetriphenylphosphonium bromide/sodium dimsylate, reagents which would lead to simultaneous homologation and oxidation.) As shown in Fig. 3, hydroboration of the alkene **4** produced the alcohol **6** in an epimeric ratio of 90:10. Ruthenium tetraoxide catalyzed oxidation of the alcohol **6** afforded the carboxylic acid **8**, which was methylated with trimethylsilyl diazomethane ($TMSCHN_2$) to yield the protected hydroxyester **10**. Removal of the *tert*-butyldimethylsilyl group with hydrogen fluoride gave the

corresponding hydroxyester **12**. As shown in Fig. 4, hydroboration of the alkene **5** yielded exclusively the alcohol **7**. Elaboration of the alcohol **7** through the acid **9** and the ester **11** yielded the lactone **20**. That the hydroxyester **11** lactonized upon deprotection confirms the stereochemical assignment of this material, as well as the *trans*-stereochemistry of the hydroxyl group in **4** and the compounds synthesized from it.

As indicated in Fig. 5, the hydroxyester **12** was then treated with 1,1'-carbonyl-diimidazole (CDI), followed by sodium azide to generate the azidocarbonate **13**. This material was immediately thermolyzed in refluxing 1,1,2,2,-tetrachloroethane (TCE), which gave the corresponding carbamate **14** in 54% yield. Only the carbamate product corresponding to the major stereoisomer at C-1 was isolated in this step. Hydrolysis of carbamate **14** in hot aqueous sodium hydroxide afforded the hydroxyamino acid **15**, which was directly coupled to the protected D-tyrosyl acid fluoride **16** to yield the protected dipeptide **17**, as a mixture of diastereomers. This mixture was coupled to D-phenylalanine benzyl ester with 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (EDC)/1-hydroxybenzotriazole (HOBt) to yield the diastereomeric, protected peptide derivatives **18a** and **18b**, which separated during chromatography on silica gel. (The derivative **15** was flanked with D-amino acids to generate a more immunogenic hapten.) Deprotection of **18** by catalytic transfer hydrogenation with palladium black and formic acid yielded the diastereomeric peptide derivatives **19a** and **19b**, which have been conjugated to the carrier proteins keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) in preparation for immunization. This synthesis is set forth in more detail in the examples below.

In summary, the synthesis of a spiro[4.4]nonane containing dipeptide analogue, which mimics both a torsionally-distorted peptide ground state and the

transition state for peptide bond hydrolysis, is described, and has been coupled at both the amino and carboxy termini of this derivative with amino acids of the D-configuration. Antibodies raised against a peptide containing this derivative can be tested for peptidolytic activity against the corresponding tetrapeptides.

The invention will be more fully described and understood with references to the following illustrated examples.

EXAMPLES

In the following examples, melting points are uncorrected. ^1H and ^{13}C NMR data were obtained on a JEOL GXS-400 spectrometer (400 MHz for ^1H NMR, 100.6 MHz for ^{13}C NMR). Chemical shifts are reported in ppm downfield from TMS at 0 ppm, and spectra are referenced with respect to the solvent peak (δ_H =7.26 ppm and δ_C = 77.0 ppm for $CDCl_3$; δ = 3.30 ppm and δ_C = 49.0 ppm for CD_3 OD). Coupling constants are reported in H_z. Infrared spectra were recorded on a Perkin-Elmer 1600 series FT-IR spectrometer. High resolution mass spectra (HRMS) were determined at the Harvard Chemistry Department Mass Spectrometry Facility. High-pressure liquid chromatography (HPLC) was performed on a Waters 600E instrument using a Waters 484 UV detector and a Waters 470 Scanning Fluorescence detector. Analytic HPLC utilized an Alltech Econosphere C18 column (15 x 0.46 cm, 5 micron); preparative HPLC utilized a Waters μBondapack Phenyl Radial-pak cartridge (10 x 2.5 cm, 10 micron). All elution solvents were CH_3CN/water mixtures containing 0.1% TFA. Column chromatography employed Aldrich silica gel 60 Å (200-400 mesh). Analytical thin-layer chromatography was performed on Baker precoated silica gel plates (Si250F), and spots were visualized with UV light and *p*-anisaldehyde stain. Chemical reagents were obtained from Aldrich or Sigma unless otherwise noted.

EXAMPLE 1

Synthesis of *cis*- and *trans*-6-Hydroxyspiro[4.4]nonane-1-one (2).

The reaction sequence is shown in Fig. 2.

The synthetic procedure of Carruthers and Orridge, J. Chem. Soc., Perkin Trans. I (1977), 2411-2416, was modified. A solution of spiro[4.4]nonane-1,6-dione **1** (47 mg, 0.309 mmol) in 1 mL of dry THF was slowly added to a stirred solution of lithium tri-*tert*-butoxyalumino-hydride (96 mg, 0.307 mmol) in 4 mL of dry THF under nitrogen at 0 °C. The solution was then warmed

to room temperature and stirred for 3 hours. The mixture was acidified with 5% acetic acid and extracted with ether (4 x 5 mL). The combined ether extracts were dried over anhydrous $MgSO_4$ and the solvent was removed by evaporation under reduced pressure. The residue was purified by chromatography on silica gel (1:1 EtOAc:hexanes, R_f 0.53) to afford **2** (37 mg, 78%) as a colorless oil; ^1H NMR showed the *trans*-isomer to be the major product (approx. 70%). IR (neat) 3446, 2957, 1733, 1161 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.16 (t, j = 6.84, 1H, *trans*-isomer), 4.01 (t, j = 3.90, 1H, *cis*-isomer), 3.37 (br s, 1H), 2.35-1.51 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 224.35, 223.98, 80.0, 59.67, 58.45, 38.29, 37.84, 35.34, 33.93, 33.72, 33.08, 32.79, 29.70, 20.79, 20.13, 19.00, 18.72; HRMS [M + NH_4] calculated for $C_9H_{18}NO_2$ 172.2474; found 172.1330.

EXAMPLE 2

Synthesis of *cis*-and *trans*-6-*tert*-Butyldimethylsililosyspiro[4.4]nonane-1-one (3).

The reaction sequence is shown in Fig. 2.

To a stirred solution of **2** (691 mg, 4.516 mmol) in 1.4 mL of dry DMF, *tert*-butyldimethylsilyl chloride (817 mg, 5.420 mmol) were added. The reaction mixture was stirred for 2 days at room temperature and then 5 mL of water was added. The mixture was extracted with ether (4 x 8 mL), the combined organic layers dried over anhydrous $MgSO_4$, and the solvent removed by evaporation under reduced pressure. Chromatography on silica gel (6:1 EtOAc:hexanes, R_f 0.68) afforded **3** as a colorless oil (1.124 g, 93%). IR (neat) 2957, 2857, 1738, 1772, 1250, 1113, 837, 776 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.15 (app. t, j =7.32, 1H, *trans*-isomer), 3.97 (dd, j = 6.47, 7.69, 1H, *cis*-isomer), 2.33-1.32 (m, 12H), 0.83 (s, 9H, *trans*-isomer), 0.82 (s, 9H, *cis*-isomer), 0.01 (s, 3H, *cis*-isomer), -0.05 (s, 3H, *trans*-isomer), -0.01 (s, 6H); ^{13}C NMR ($CDCl^3$): δ 222.44, 219.52, 82.46, 77.24, 59.64, 58.09, 38.26, 38.18, 36.61, 34.03, 33.85, 33.33, 32.19, 29.96, 25.33, 20.69, 20.16, 19.29, 19.03, 17.50, 17.38, -5.11, -

5.47, -5.50; HRMS [M + H] $^+$ calculated for $C_{15}H_{29}O_2Si$ 269.1937, found 269.1940.

EXAMPLE 3

Synthesis of *trans*-*tert*-Butyldimethylsilyloxyspiro[4.4] nonane-6-ene (4) and *cis*-*tert*-Butyldimethylsilyl-oxyspiro[4.4]nonane-6-ene (5).

The reaction sequence is shown in Fig. 2.

Sodium hydride (24 mmol as a 60% dispersion in mineral oil) was washed with 50 mL of anhydrous ether under nitrogen, and 10 mL of dry DMSO was added. The mixture was heated at 70-75 °C for approximately 1 hour until a clear dark-gray solution formed. The resulting solution was cooled to 0 °C, and methyltriphenylphosphonium bromide (9.0 g, 25,193 mmol) in 25 mL of dry DMSO was added. The resulting dark-red solution was stirred at room temperature for 15 minutes and then **3** (680 mg, 2.537 mmol) in 5 mL of DMSO was added. The reaction mixture was stirred at 55 °C for 3 hours, cooled, and diluted with water. The resulting mixture was extracted with EtOAc (4 x 40 mL), and the combined extracts were washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed by evaporation under reduced pressure, and the residue purified by chromatography on silica gel (hexanes, R_f **4** 0.39, R_f **5** 0.68) to afford **4** (316 mg, 47%) and **5** (160 mg, 24%) as colorless oils. **4**: IR (neat) 3077, 2955, 2856, 1646, 1472, 1256, 1122, 877, 836, 775 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.91 (app. t, j = 1.96, 1H), 4.77 (app. t, j = 2.08, 1H), 3.98 (app. t, j = 7.20, 1H), 2.36 (m, 2H), 2.12 (m, 1H), 1.96 (m, 1H), 1.64-1.38 (m, 8H), 0.90 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C ($CDCl_3$) δ 159.58, 102.98, 80.46, 56.96, 37.74, 34.66, 32.96, 32.72, 25.88, 23.47, 20.13, 18.04, -4.58, -4.81; HRMS [M + H]$^+$ calculated for $C_{16}H_{31}O_6Si$ 267.2144, found 267.2149. **5**: IR (neat) 3085, 2954, 2856, 1653, 1472, 1254, 1113, 1057, 835, 773 cm^{-1}; ^1H NMR ($CDCl_3$) δ4.97 (m, 1H), 4.88 (m, 1H), 3.66 (dd, j = 2.56, 5.0, 1H), 2.35 (m, 2H), 1.94 (m, 2H), 1.79 (m, 1H), 1.38-1.67 (m, 7H), 0.87 (s, 3H), -0.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ

153.78, 107.55, 79.47, 57.70, 39.39, 36.37, 34.56, 32.79, 26.01, 22.99, 20.76, 18.24, -4.56, -4.76; HRMS $[M + H]^+$ calculated for $C_{16}H_{31}O_6Si$ 267.2144, found 267.2131.

EXAMPLE 4

Synthesis of *trans*-1-*tert*-Butyldimethysilyloxy-*trans*-6-hydroxymethyspiro[4.4]nonane and *trans*-1-*tert*-Butyldimethysilyloxy-*cis*-6-hydroxymethylspiro[4.4]nonane(6).

The reaction sequence is shown in Fig. 3.

To a stirred solution of **4** (362 mg, 1.356 mmol) at 0 °C in 27 mL of dry THF under an atmosphere of nitrogen was added 1M borane.THF complex (2.7 mL, 2.700 mmol). The reaction mixture was maintained at 0 °C for 30 min and then stirred at room temperature for another 30 min. The solution was cooled to 0 °C again. After successive additions of 3N aqueous NaOH (4 mL) and 30% hydrogen peroxide (4 mL), the reaction mixture was stirred at 0 °C for 1 h. The solution was concentrated to one-half its volume by evaporation under reduced pressure and diluted with 10 mL of water. The solution was extracted with EtOAc (4 x 25 mL), and the combined extracts washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed by evaporation under reduced pressure, and the residue purified by chromatography on silica gel (4:1 hexanes:EtOAc, R_f0.53) to give **6** as a colorless, viscous oil (336 mg, 87%). ^1H NMR showed **6** to be a 9:1 ratio of diastereomers. IR (neat) 3347,2957, 2862, 1463, 1256, 1109, 1027, 881, 836, 774 cm^{-1}; ^1H NMR ($CDCl_3$) δ4.09 (app. t, j = 7.70, 1H, major), 3.83 (app.t, j = 7.33, 1H, minor), 3.62 (m, 2H, major), 3.48 (m, 2H, minor), 2.49 (br s, 1H), 1.90-1.32 (m, 13H), 0.88 (s, 9H), 0.08 (s, 3H, major), 0.06 (s,3H, minor), 0.02 (s, 3H, minor); ^{13}C NMR ($CDCl_3$)δ76.68, 76.1, 64,34, 64,0, 54,86, 49.34, 46.0, 36.86, 32.88, 31.93, 31.93, 31.80, 30.80, 29.75, 29.14, 25.83, 25.80, 23.07, 22.66, 19.03, 17.92, -3.37, -4.35, -4.81, -5.00; HRMS $[M+H]^+$ calculated for $C_{16}H_{33}O_2Si$ 285.2250, found 285.2240.

EXAMPLE 5

Synthesis of *trans*-6-*tert*-Butyldimethylsiloxyspiro[4.4] nonane-*trans*-1-carboxylic acid and *trans*-6-*tert*-Butyldimethylsilyloxyspiro[4.4]nonane-*cis*-1-carboxylic acid (8).

The reaction sequence is shown in Fig. 3.

To a solution of 6 (210 mg, 0.739 mmol) and sodium periodate (514 mg, 2.403 mmol) in a mixture of 1.5 mL of CCl_4, 1.5 mL or CH_3CN, and 2.25 mL of water was added ruthenium trichloride hydrate (4 mg, 0.019 mmol). The reaction mixture was stirred vigorously for 3 hours at room temperature, and 10 mL of CH_2Cl_2 was added. The upper aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL), the combined organic extracts dried over anhydrous $MgSO_4$, and the solvent removed by evaporation under reduced pressure. The resulting residue was diluted with 120 mL of ether, and the solution filtered through a small silica gel column to remove the ruthenium trichloride. The filtrate was dried over anhydrous $MgSO_4$, and the solvent removed by evaporation under reduced pressure to afford 8 (203 mg, 92%) as a viscous, colorless oil. IR (neat) 3500-2450, 2956, 2857, 1703, 1699, 1471, 1422, 1250, 1112, 837, 775 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.05 (brs, 1H), 4.05 (app. t, J=6.0, 1H), 2.69 (app. t, J=8.55, 1H, minor), 2.52 (app. t, J=7.69, 1H, major), 2.12-2.20 (m, 1H, 2.06-1.38 (m, 11H), 0.88 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 181.95, 76.53, 57.95, 51.83, 36.58, 33.76, 32.61, 28.72, 25.84, 23.05, 19.95, 17.92, -4.05, -5.11; HRMS[M - H]$^-$ calculated for $C_{16}H_{29}O_3Si$ 297.1886, found 297.1891.

EXAMPLE 6

Synthesis of Methyl *trans*-6-*tert*-Butyldimethylsilyloxyspiro[4.4]nonane-*trans*-1-carboxylate and Methyl *trans*-6-*tert*-Butyldimethylsilyloxyspiro[4.4]nonane-*cis*-1-carboxylate (10).

The reaction sequence is shown in Fig. 3.

To a stirred solution of 8 (157 mg, 0.524 mmol) in a mixture of 3.5 mL hexanes and 1.0 mL CH_3OH was added trimethylsilydiazomethane (500 mL, 2M solution in

hexanes) at room temperature. The mixture was stirred for 1 hour at room temperature, and the solvents removed by evaporation under reduced pressure to give **10** as a colorless liquid (164 mg, 100%). R_f0.64 (6:1 hexanes:EtOAc). IR (neat) 2995, 2857, 1732, 1463, 1434, 1257, 1111, 837, 775 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.96 (app. t, J=5.25, 1H, major), 3.63 (s,3H), 2.62 (app. t, J=8.54, 1H, minor), 2.46(app.t,J=7.69, 1H, major), 2.14-1.24(m, 12H), 0.87(s, 9H, minor), 0.84(s, 9H, major), 0.04(s, 3H, minor), 0.02 (s, 3H, major), -0.01 (s, 3H, major); ^{13}C NMR ($CDCl_3\delta$ 175.26, 58.19, 51.84, 51.05, 36.50, 33.93, 33.06, 28.72, 25.78, 23.02, 20.21, 17.90, -4.19, -5.10; HRMS [M + H]$^+$ calculated for $C_{17}H_{33}O_3Si$ 313.2199, found 313.2205.

EXAMPLE 7

Synthesis of Methyl *trans*-6-Hydroxyspiro[4.4]nonane-*trans*-1-carboxylate and Methyl *trans*-6-Hydroxyspiro[4.4]nonane-*cis*-1-carboxylate (12).

The reaction sequence is shown in Fig. 3.

To a stirred solution of **10** (210 mg, 0.673mmol) in 3mL of CH_3CN at 0°C, two drops of 50% of hydrogen fluoride were added. The reaction was stirred at room temperature for 2 h and 5 mL of water was added. The mixture was extracted with $CH2Cl_2$ (4 x 10mL), and the combined extracts were dried over anhydrous $MgSO_4$. The solvent was removed by evaporation under reduced pressure to afford **12** as a colorless, viscous oil (130 mg, 98%). R_f0.53 (major), 0.44 (minor) (2:1 hexanes:EtOAc). IR (neat) 3503, 2957, 2872, 1731, 1436, 1289, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.94 (app. t, J=7.32, 1H, minor), 3.78 (app. t,J=7.56, 1H, major), 3.58(d,J=7.45, 1H, major), 2.07-1.37 (m,12H); ^{13}C NMR ($CDCl_3$ δ177.30, 76.32, 56.30, 52.20, 37.6\52, 37.53, 31.32, 30.58, 30.30, 23.78, 18.72; HRMS [M + H$^+$ calculated for $C_{11}H_{11}O_3$ 199.2701, found 199.1344.

EXAMPLE 8

Synthesis of Methyl Spiro-*trans*-1-oxa-2-oxo-*trans*-3-aza-bicyclo[3.3.0]octane-7,2'-cyclopentane-1'-carboxylate (14).

The reaction sequence is shown in Fig. 5.

To a solution of **12** (125 mg, 0.631 mmol) in 2mL of benzene, 1,1'-carbonyldiimidazole (205 mg, 1.264 mmol) and pyridine (0.153 mL, 1.892 mmol) were added. The reaction mixture was stirred at room temperature of 3 h and then 10mL of EtOAc was added. The resulting solution was washed quickly with brine (3 x 5 mL), the organic phase dried over anhydrous $MgSO_4$, and the solvent removed by evaporation under reduced pressure to give a clear oil. To this coil, 3 mL of dry DMF and sodium azide (205 mg, 3.153 mmol) were added. The reaction medium was then acidified to approximately pH4 with concentrated HCl and stirred at room temperature overnight. 15mL of brine was added, and the aqueous layer was extracted with EtOAc4 x 20 mL). The combined organic layers were washed with brine (2x10 mL), dried over anhydrous $MgSO_4$, and the solvent removed by evaporation under reduced pressure to afford the azidoformate **13** as a colorless oil (156 mg, yield 92%). [^{1}H NMR ($CDCl_3$) δ 4.98 (dd,J=5.62, 6.83, 1H minor), 4.88 (dd, J=4.76, 6.71, 1H major), 3.60 (s, 3H), 2.61 (app.t, J=8.3, 1H), 2.48 (dd, J=6.35, 7.57, 1H), 2.10-1.40 (m,12H); ^{13}C NMR ($CDCl_3$) 174.66, 156.64, 83.15, 56.75, 51.60, 51.42, 36.61, 31.87, 30.35, 28.28, 22.73, 19.74.] A solution of **13** (103 mg, 0.386 mmol) in 30 mL of 1,1,2,2-tetrachloroethane (TCE) was added to an additional 220 mL of refluxing TCE. The solution was refluxed for 45 min. and the solvent was removed by evaporation under reduced pressure to yield a brown oil, which was purified by chromatography on silica gel (EtOAc,R_f0.53) to afford **14** as a waxy solid (50 mg, 54%). IR (film) 3277, 2956, 2874, 1766-1716, 1435, 1252, 1162, 1047 cm^{-1}; ^{1}H NMR ($CDCl_3$ δ 6.33 (brs, 1H), 4.84 (d, J=7.08, 1H), 4.20 (app. t, J=6.1,1H), 3.67(s,3H),2.51, (app.t,J=8.18,1H), 2.19-1.59 (m,10H); ^{13}C NMR ($CDCl_3$)δ 175.20, 159.63, 84.70, 58.75, 56.96, 52.29, 51.73, 34.73, 33.64, 32.35, 29.46, 22.89, HRMS [M + H]$\pm$ calculated for $C_{12}H_{18}NO_4$ 240.1236, found 240.1235.

EXAMPLE 9

Synthesis of *N*-(*N*-(Carbobenzyloxy)-*O*-benzyl-D-tyrosine Carboxylic Acid Fluoride (16).

To solution of Cbz-D-Tyr(OBz)-H [BACHEM Bioscience Inc.] (1.62 g, 4.000 mmol) in Ch_2Cl_2(10mL) under argon at -10°C was added pyridine (0.324mL, 4007 mmol) and cyanuric fluoride [Fluka Chemie AG] (1.08 g, 7.997 mmol). After 75 min the reaction was quenched by the addition of crushed ice, and an additional 20 mL of CH_2Cl_2 was added. The organic layer was then removed, and the aqueous layer extracted with CH_2Cl_2(10mL). The combined organic layers were washed with ice-cold water (10mL), dried over anhydrous $MgSO_4$, and solvent removed by evaporation under reduced pressure. The yellow residue was recrystallized from hexanes to afford **16** (1.08 g, 72%) as white solid (mp 79-80°C). IR ($CHCl_3$) 3431, 3034, 1845, 1723, 1512, 1244; ^1H NMR ($CDCl_3$ δ7.42-7.30 (m,10H), 7.10-7.07 (d,*J*=8.0Hz, 2H),6.96-6.93 (d,*J*=8.0Hz,2H), 5.14(s, broad, 1H, 5.12 (s,2H), 4.81-4.75(m,1H), 3.14-3.10 (d,*J*=5.75Hz,2H); ^{13}C NMR($CDCl_3$) δ161.89(d,$^1J_{C-F}$=363.6Hz), 158.39,1, 155.51, 136.77, 135.72, 130.35, 128.59, 128.33, 128.14, 128.03, 127.52, 126.28, 115.36, 70.06, 67.40, 53.82 (d,$^2J_{C-F}$=58.1 Hz), 36.00; HRMS (M+Na)$^+$ calculated for $C_{24}H_{22}O_4NF$ 407.1533, found 407.1535.

EXAMPLE 10

Synthesis of *N*[(*N*-(Benzyloxycarbonyl-*O*-benzyl)-D-tyrosyl)-7-*trans*-amino-6-*trans*-hydroxyspiro[4.4]nonane-1-Carboxylic Acid (As a Mixture of Diastereomers) (17).

The reaction sequence is shown in Fig. 5.

A solution of **14** (19 mg, 0.079 mmol) in 0.4 M aqueous sodium hydroxide (2mL) was heated at 75°C for 18 hours. After cooling, the reaction mixture was neutralized with 1N HCl. The solvent was removed by evaporation under reduced pressure, and the residue taken up in acetone. The slurry was filtered to remove sodium chloride, and the solvent removed by evaporation under reduced pressure to yield the deprotected derivative **15** (approximately 100% by ^1H NMR). [^1H NMR(CD_3OD):δ3.97

(δ,*J*=4.88,1H), 3.70(m,1H), 2.49(app.t,*J*=5.50,1H),2.18-1.43(m,10H); ^{13}C NMR (CD3OD): δ179.22, 75.26, 59.46, 54.64, 54.05, 35.87, 34.40, 29.56, 27.65, 23.47.] To a stirred solution of the derivative **15** in 2 mL of dry DMF at 0°C under nitrogen was added pyridine 15 μl, 0.185 mmol). The acid fluoride **16** (35 mg, 0.086 mmol) in 0.5 mL of dry DMF was then added dropwise. The solution was stirred in 0°C, and the reaction monitored by analytical HPLC (CH_3CN 45% for 2 min; 45-70% over 28 min; 70-100% over 5 min, flow rate: 1 mL/min; t_R=14.67 and 15.23 minutes for the two diastereomers). After 30 minutes, the solvents were removed by evaporation under reduced pressure, and the residue dissolved in a minimum of CH_3CH/water. The products were purified by preparative HPLC(CH_3CH:45% for 2 min; 45-60% over 30 min; 60-100% over 10 min; flow rate:7mL/min) to yield **17** (25mg, 54%) as a mixture of diastereomers. ^{1}H NMR (CD_3OC)δ7.42-7.22(m,10H), 7.12(d,*J*=7.57,2H), 6.89(d,*J*=7.562H),5.02(s,4H), 4.32-4.15(m,2H), 3.83 (dd, *J*=5.01, 25.52, 1H), 3.04 (ddd, *J*=5.86, 13.91, 27.83, 1H), 2.78 (m, 1H),2.48(dd,*J*=6.59, 14.65, 1H), 2.13 (m,2H), 1.94(m,2H), 1.79(m,1H), 1.61-1.41 (m,5H); ^{13}C NMR (CD_3OD)δ 179.58, 174.22, 159.95, 139.63,132.15, 131.72, 131.54, 130.28, 130.25, 130.18, 129.72, 129.62, 129.46, 129.31, 116.73, 77.48, 71.81, 68.37, 59.84, 59.70, 55.0, 54.87, 54.61, 39.18, 38.61, 37.0, 35.35, 30.31, 30.10, 24.25; HRMS[M - H$^-$ calculated for $C_{34}H_{37}N_2O_7$ 585.2601, found 585.2609.

EXAMPLE 11

Synthesis of *N-[N-*(Benzyloxycarbonyl-*O*-benzyl)-D-tryosyl]-7-hydroxyspiro[4.4]nonane amino-*trans*-6-nonane-1-carboxoyl-D-phenylalanine Benzyl Ester (Separated into Diastereomers 18a and 18b).

The reaction sequence is shown in Fig. 5.

To a stirred solution of **16** (24mg, 0.041 mmol), 1-hydroxybenzo-triazole (10mg, 0.074 mmol), and H-D-Phe-OBz (11mg, 0.043 mmol) in 4 mL of dry THF at 0°C under nitrogen, was added 1-ethyl-3-(3-

dimethylaminopropyl)carbodiimide HCl(14 mg, 0.073 mmol). (H-D-Phe-OBz was prepared by partitioning H-D-Phe-OBz-*p*-tosylate [BACHEM Biosciences Inc.] between 5% sodium bicarbonate and EtOAc. The organic layer was separated and dried over anhydrous $MgSO_4$, and the solvent was removed by evaporation under reduced pressure.) The resulting solution was stirred at 0°C for 1 h and then overnight at room temperature. The solvent was removed by evaporation under reduced pressure, and the residue purified by chromatography on silica gel (2:1 EtOAc:hexanes Rf **18a** 0,55, R_f **18b** 0.42) to afford **18a**(14 mg, 41%) and **18b** (14mg, 41%). **18a**: 1H NMR ($CDCl_3$)δ7.43-7.24 (m,12H), 7.10-7.04 (m, 5H, 6.87 (d, *J*=4.67, 2H), 6.44 (d,*J*=4.64, 1H), 6.0 (d, *J*=8.05, 1H), 5.31, (d, *J*=7.57, 1H), 5.17 (q, *J*=11.96, 2H), 5.08 (d, *J*=4.64, 2H), 5.01 (s,2H), 4.96 (dt, *J*=5.86, 7.81, 1H), 4.35 (m,1H) 3.90 (m,1H), 3.42 (d,*J*=7.82, 1H), 3.17 (dd,*J*=5.62, 13.92, 1H) 3.0 (m,3H), 2.24 (app.t,*J*=7.57,1H), 2.07 (m,1H) 1.9\88 (m,2H), 1.71 (m,.2H, 1.48 (m,3H), 1.15 (m,2H); ^{13}C NMR ($CDCl_3$)δ 175.62, 171.55, 170.38, 157.85, 155.72, 136.99, 136.30, 135.55, 134.91, 130.42, 130.35, 130.32, 129.17, 128.67, 128.63, 128.51, 128.12, 128.04, 128.01, 127.96, 127.93, 127.46, 127.41, 127.29, 114.95, 76.55., 74.03, 70.0, 67.49, 66.88, 55.88, 55.38, 52.88, 51.33, 38.04, 36.63, 31.25, 30.0, 24.21, HRMS [M + Ma]$^+$ calculated for $C_{60}H_{53}N_3O_8Na$ 846.3730, found 846.3705. **18b**: ^1H NMR($CDCl_3$)δ7.43-7.21 (m,13H), 7.12-6.96 (m,4H), 6.89 (d*J*=8.55, 2H), 6.33 (d,*J*=4.88, 1H), 6.0 (d, *J*=7.57, 1H), 5.16 (q, *J*=11.96, 2H), 5.08 (s, 2H), 5.01 (s, 2H), 4.86 (dt,*J*=7.32, 5.98, 1H), 4.31 (m, 1H), 3.98(m,1H), 3.83 (d,*J*=7.81, 1H), 3.16-2.93 (m,4H), 2.23 (app.t,*J*=7.33,1H), 2.04 (m,1H), 1.74 (m,4H), 1.43-1.21(m,5H); ^{13}C NMR ($CDCl_3$δ 176.02, 171.27, 170.27, 157.82, 155.64, 136.97, 135.38, 134.86, 130.35, 129.21, 128.67, 128.61, 128.56, 128.50, 128.12, 128.09, 128.01, 127.96, 127.46, 127.43, 127.20, 114.92, 73.92, 69.93, 67.54, 66.84, 56.04, 55.41, 53.41, 53.04, 53.04, 51.49, 37.42, 36.84, 31.38, 30.67, 24.13;

HRMS [M+ Na]$^+$calculated for $C_{60}H_{53}N_3O_8Na$ 846.3730, found 846.3713.

EXAMPLE 12

Synthesis of *N*-(D-Tyrosyl)-*trans*-7-amino-*trans*-6-hydroxyspiro[4.4]nonane-1-carboxoyl-D-phenylalanine (Diastereomer 19a).

The reaction sequence is shown in Fig. 5.

To a solution of **18a** (30mg, 0.036 mmol) in 1.5 mL of 10% formic acid in THF was added palladium black (32 mg in 1.5 mL of water). The reaction mixture was stirred for 20 minutes, at which time analytical HPLC showed quantitative conversion to product (CH_3CN:45% for 2 minutes, 45-70% over 28 min, 70-100% over 10 min, flow rate; 1 mL/min; t_r=6.44 min). The catalyst was removed by filtration, and the solvents removed by evaporation under reduced pressure to afford the pure deprotected peptide **19a** (20mg, 100%). ^{1}H NMR (CD_3OD) δ8.17(s,1H), 7.29-7.03(m,7H), 6.78-6.68(m,2H), 4.68(m,1H), 4.16-3.47(m,3H), 3.42(m,1H), 3.20(m,1H), 2.95(m,2H) 2.40 (m,1H), 2.08-1.24(m, 10H); ^{13}C NMR (CD_3OD) 177.23, 169.14, 165.14, 165.77, 158.22, 138.81, 131.63, 130.42, 129.50, 127.75, 126.20, 116.87, 116.77, 75.66, 58.12, 55.83, 55.49, 55.17, 53.60, 38.71, 38.12, 36.39, 34.14, 30.79, 29.69, 24.34; HRMS [M + H$^+$ calculated for $C_{28}H_{36}N_3O_6$510.2604, found 510.2608.

EXAMPLE 13

Synthesis of Diastereomer 19b

Diastereomer **19b** was prepared from **18b** exactly as described in Example 11 above. HPLC t_r = 8.62 min; ^{1}H MNR(CD_3)D) δ8.24(s,1H, 7.27-7.12(m, 5H), 7.07(d,J=8.05, 2H), 6.74(d,J=8.3,2H), 4.59 (dd,J=4.64,9,57,1H), 4.16 (m,1H), 4.04(m,1H), 3.96 (d,J=5.86,1H), 3.22(m,1H), 2.98-2.86 (m,3H), 2.35 (m,1H), 1.96 (m,2H), 1.74 (m,2H), 1.55 (m,3H, 1-44-1.17 (m,3H); ^{13}C NMR (CD_3OD) δ177.33, 169,35, 166.61, 158.19, 139.15, 131.63, 130.27, 129.27, 127.51, 126.30, 116.30, 116.85, 75.84, 58.64, 58.04, 55.83, 55.06, 53.91, 53.59, 38.78, 37.92, 36.63, 34.11, 33.22,

29.95, 29.24, 24.00; HRMS [M + H]$^+$ calculated for $C_{28}H_{36}N_3O_6$ 510.2604, found 510.2618.

EXAMPLE 14

Production, Screening and Isolation of Monoclonal Antibodies Against Diastereomers 19a and 19b

Diastereomers 19a and 19b are prepared as described in Examples 12 and 13, and are then conjugated to the carrier protein keyhole limpet hemocyanin (KLH). BALB/c mice are immunized with the KLH-conjugated compounds emulsified in complete Freund's adjuvant. A blood sample is obtained from each mouse and the serum separated by centrifugation and stored at 4°C. Sera obtained in this way are screened for binding activity to the original transition-state analog immunogen by standard ELISA procedures. Antibody-producing mice immunized as described above and assayed for reactivity with the transition state analog peptide immunogens are sacrificed, their spleens are removed and hybidoma cells are prepared using myeloma cells. Hybidomas secreting monoclonal antibodies are screened for hydrolytic activity against the peptide substrates D-tyrosyl-glycyl-glycyl-D-phenylalanine, D-tyrosyl-glycyl-D-prolyl-D-phenylalanine, and D-tyrosyl-glycyl-L-prolyl-D-phenylalanine.

These methods are well known to one of ordinary skill in the art, and are detailed in the parent application Serial No. 08/134,492, filed October 8, 1993.

Although certain presently preferred embodiments of the invention have been described herein, it will be apparent to those skilled in the art to which the invention pertains that variations and modifications of the described embodiment may be made without departing from the spirit and scope of the invention. Accordingly, it is intended that the invention be limited only to the extent required by the appended claims and the applicable rules of law.

REFERENCES

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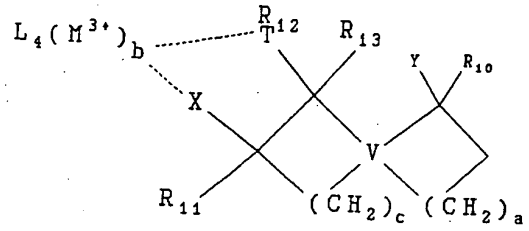
3. A. Tramontano et al., "Chemical Reactivity at an Antibody Binding Site Elicited By Mechanistic Design of a Synthetic Antigen", Proc. Natl. Acad, Sci, USA, 83, 6736-6740 (1986).
4. H. White and W.P. Jencks, J. Bio. Chem., 251, p. 1688 (1976); H. White et al., ibid, 1700.
5. W.P. Jencks, Symposia on Quantitative Biology, 52, 65 (1987).
6. H.M. Geysen et al., J. Immunological Methods, 102, 259-274 (1987).
7. H.M. Geysen et al., Proc. Nat'l. Acad. Sci, USA, 82 178-182 (1985).
8. J.A. Berzofsky, Science, 229, 932-940 (1985).
9. T.P. Hopp and K.R. Woods, Proc. Nat'l. Acad. Sci, USA, 78, 3824-3828 (1981).
10. J. Novotny et al., Proc. Nat'l. Acad. Sci., 226 (1986).
11. H.M. Geysen et al., Science, 235, 1184 (1987).

WHAT IS CLAIMED IS:

1. An hapten of formula I



XVIII

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 0 to 10;

R_{10} and R_{11} may be the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

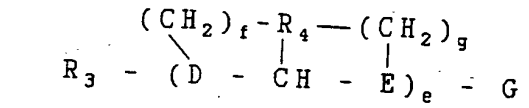
L is a ligand;

M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N^+ with any negatively-charged counterion, or C;

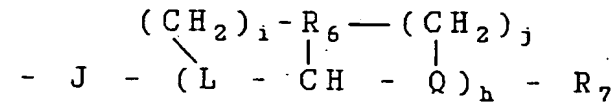
X is OH, SH, NH_2, NH_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or $O(C_1-C_{10})$alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9) alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, or (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

and wherein

one or more of R_{10}, R_{11}, R_4 and R_6 are unbound or bound to one or more of said remaining substituents R_{10}, R_{11}, R_4 and R_6 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$, and $-(CH_2)_u-phenyl-(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10.

2. The hapten of claim 1, wherein:

V is C;

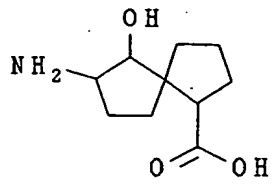
X is NH_2;

Y is COR_5;

a and c are both 2; and

R_{10}, R_{11}, R_{12}, and R_{13} are all hydrogen.

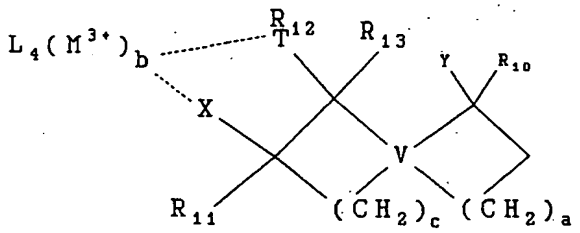
3. The hapten of claim 2, wherein said hapten has the formula:



4. A catalytic antibody elicited by an hapten of claim 1.

5. An immunogen comprising:

(a) a hapten of formula XVIII



XVIII

or a physiologically acceptable salt thereof, wherein:

a is an integer from 0 to 10;

b is 0 or 1;

c is an integer from 0 to 10;

R_{10} and R_{11} may be the same or different and each is a side chain of a naturally occurring amino acid or an analog of said side chain;

R_{12} is hydrogen or a second bond between T and the carbon to which T is attached provided that if R_{12} is a second bond, then there is no substituent R_{13};

R_{13} is hydrogen;

L is a ligand;

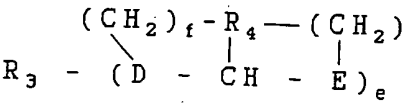
M^{3+} is Cr(III) or Co(III);

T is O or S;

V is N^+ with any negatively-charged counterion, or C;

X is OH, SH, NH_2, HN_2 protected by a protecting group selected from the group consisting of terminal amino protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9)alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio,

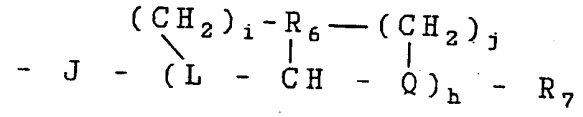
phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, (C_1-C_4)alkoxycarbonyl, or



e is an integer from 1 to 10;

f and g are 0 or 2 provided f and g are not both 2;

Y is hydrogen, COR_5, carboxyl protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, (C_1-C_9)alkyl, (C_1-C_9) alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di- or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, or (C_1-C_4)alkoxycarbonyl or



h is an integer from 1 to 10;

i and j are 0 or 2 provided i and j are not both 2;

R_3 is hydrogen or a protecting group selected from the group consisting of amino-terminal and carboxyl-terminal protecting groups;

R_4, being the same or not all the same when e > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_4 is CH_2 when f or g is 2;

R_5 is OH, NH_2 or O(C_1-C_{10})alkyl;

R_6, being the same or not all the same when h > 1, is a side chain of a naturally occurring amino acid or an analog of said side chain provided that R_6 is CH_2 and when i or j is 2;

R_7 is OH, SH, NH_2, OH protected by a protecting group selected from the group consisting of terminal carboxyl protecting groups, alkene, (C_1-C_9)alkyl, (C_1-C_9) alkoxy, phenyl, phenoxy, cyclohexyl, phenylthio, phenylsulfinyl or phenylsulfonyl wherein the aforementioned phenyl groups are unsubstituted or mono-, di-or trisubstituted by halogen, (C_1-C_4)alkyl, (C_1-C_4)alkoxy, or (C_1-C_4)alkoxycarbonyl;

D and E are the same or different when e is 1 and are the same or not all the same when e > 1 and: when f and g are 0, each of D and E is NH, O, S, CH_2, CF_2, C=O or C=S; when f is 2, D is N, CH or CF; when g is 2, E is N, CH or CF; and when e > 1 and when D and E are directly adjacent to each other, then D and E are CH or N and are joined by a double bond provided that D or E is C when f or g is 2;

G is NH, O, S, CH_2, CF_2, C=O or C=S;

J is NH, O, S, CH_2, CF_2, C=O or C=S;

L and Q are the same or different when h is 1 and are the same or not all the same when h > 1 and: when i and j are 0, each of L and Q is NH, O, S, CH_2, CF_2, C=O or C=S; when i is 2, L is N, CH or CF; when j is 2, Q is adjacent to each other, L and Q are CH or N and are joined by a double bond provided that L or Q is C when i or j is 2;

and wherein

one or more of R_{10}, R_{11}, R_4 and R_6 are unbound or bound to one or more of said remaining substituents R_{10}, R_{11}, R_4 and R_6 provided R_4 is unbound to said remaining substituents when f or g is 2 and R_6 is unbound to said remaining substituents when i or j is 2, and if the aforementioned groups are bound to one another, then by a covalent bond or a linker moiety selected from the group consisting of $-(CH_2)_u-S-S-(CH_2)_v-$, $-(CH_2)_v-$, $-S-(CH_2)_v-S-$, $-(CH_2)_u-S-(CH_2)_v-$, $-(CH_2)_u-CH=CH-(CH_2)_v-$, $-(CH_2)_u-NH-CO-(CH_2)_v-$, $-(CH_2)_u-NH-(CH_2)_v-$, and $-(CH_2)_u-$phenyl$-(CH_2)_v-$; and

u and v are the same or different and each is 0 or an integer from 1 to 10 unless the linker moiety is $-(CH_2)_v-$ in which case v is an integer from 1 to 10; and

(b) a carrier molecule, said hapten being coupled to said carrier molecule by a suitable coupling moiety.

6. The immunogen of claim 5, wherein:

V is C;

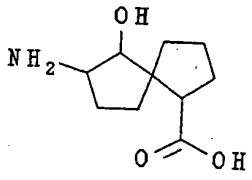
X is NH_2;

Y is COR_5;

a and c are both 2; and

R_{10}, R_{11}, R_{12} and R_{13} are all hydrogen.

7. The immunogen of claim 6, wherein said hapten has the formula:



8. A catalytic antibody elicited by an immunogen of claim 5.

9. A catalytic antibody which can catalyze a chemical reaction of interest and which is elicited through in vitro or in vivo techniques by an antigen comprising a hapten as in claim 1, said catalytic antibody having been prepared by a process comprising the steps of:

(a) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

(b) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(c) screening said plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes said chemical reaction of interest.

10. A method for producing catalytic antibodies which can catalyze a chemical reaction of interest and which are elicited through in vitro or in vivo techniques by an antigen comprising a hapten as in claim 1, wherein said method comprises the steps of:

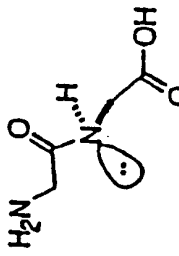
(a) exposing cells capable of producing antibodies to said antigen and thereby generating antibody producing cells;

(b) hybridizing said antibody producing cells with myeloma cells and thereby generating a plurality of hybridoma cells each producing monoclonal antibodies; and

(c) screening said plurality of monoclonal antibodies to identify a monoclonal antibody which catalyzes said chemical reaction of interest.

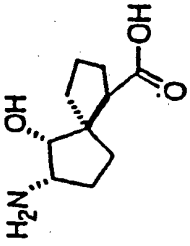
11. A method for catalyzing the cleavage or formation of a peptide bond in a molecule comprising the step of contacting said molecule with an effective amount of a catalytic antibody elicited by an antigen comprising a hapten as in claim 1.

FIG. 1c



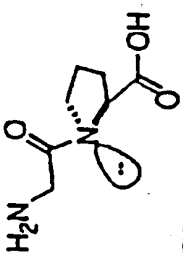
TORSIONALLY-DISTORTED GLYCYL-GLYCINE

FIG. 1b



SPIRO ANALOGUE

FIG. 1a



TORSIONALLY-DISTORTED GLYCYL-PROLINE

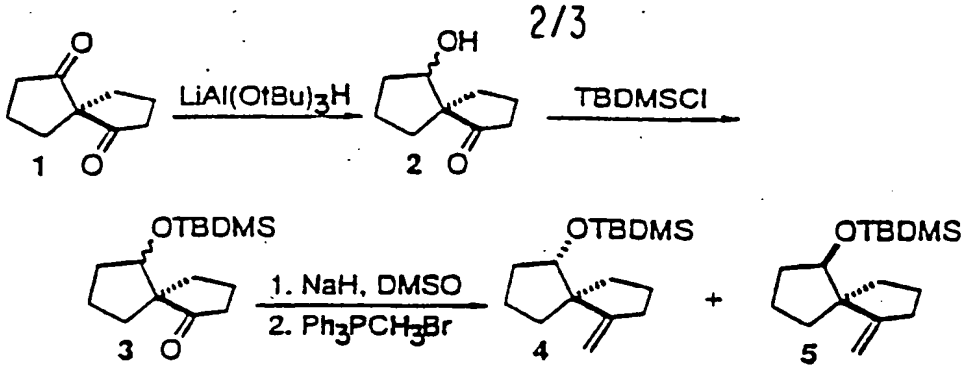


FIG. 2

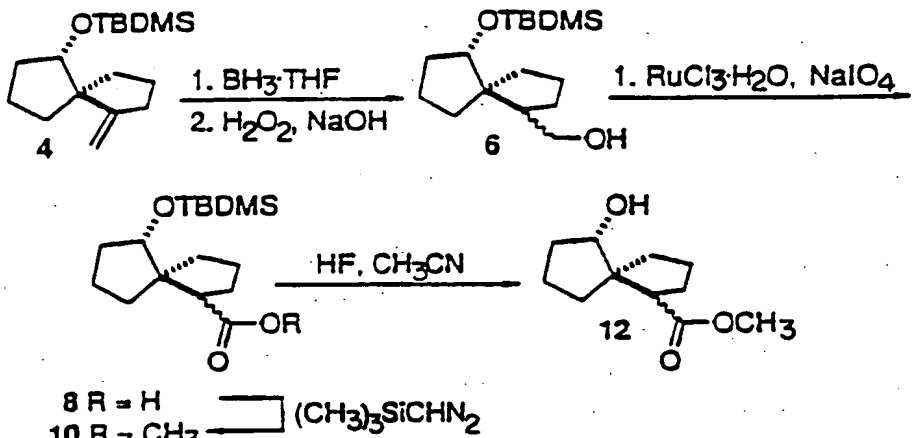


FIG. 3

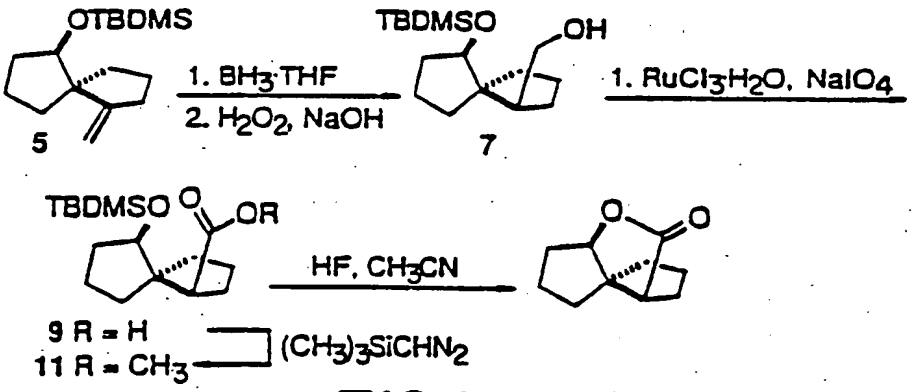


FIG.4

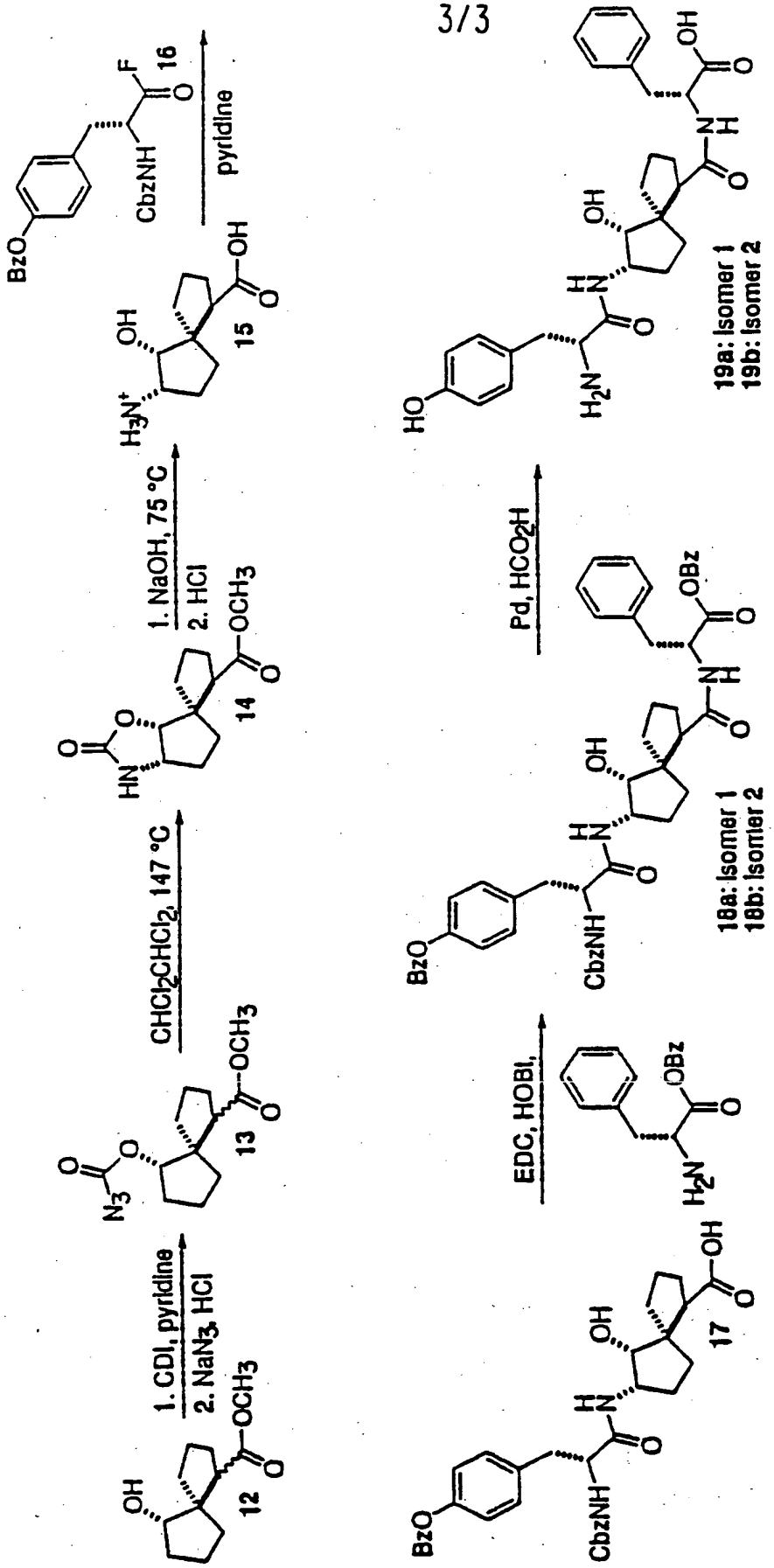

1. CDI, pyridine
2. NaN3, HCl
CHCl2CHCl2, 147 °C
1. NaOH, 75 °C
2. HCl
pyridine
EDC, HOBt,
Pd, HCO2H
12
13
14
15
16
17
18a: isomer 1
18b: isomer 2
19a: isomer 1
19b: isomer 2

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09450

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07K 16/44; C07C 211/00
US CL : 435/188.5; 564/460
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/188.5; 564/457, 460

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database Medline on STN, US National Library of Medicine, (Bethesda, MD, USA), No. 95031013, SMITH et al. 'An Approach to Sequence-Specific Antibody Proteases. The Use of Haptens Mimicking Both a Transition State and a Distorted Ground State,' abstract 95031013, Applied Biochemistry and Biotechnology, May-June 1994, Volume 47, Number 2-3, see entire document.	1-11
Y	YUAN et al. The Synthesis of Cyclobutanol-Containing Dipeptide Analogues. Tetrahedron Letters. 1994, Vol. 35, No.34, pages 6195-6198, see entire document.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 SEPTEMBER 1996	Date of mailing of the international search report 16 SEP 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Deborah Frieso for MICHAEL P. WOODWARD Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09450

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YUAN et al. A Mimic of Both a Torsionally-Distorted Peptide Ground State and the Transition State for Peptide Bond Hydrolysis: Synthesis of a Spiro[4.4]nonyl Derivative. Journal Org. Chem. 1995, Vol. 60, No. 16, pages 5360-5364.	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/09450

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, MEDLINE, CAS
search terms: nonane,564,transition, peptide or protease,spiro; glycyl, glycine, proline, transition, catalytic antibo?,protease, cleav?, HIV, Wolfenden, Radzicka, Hansen, Yuan; renin, transition state, nonane